Mesenchymal stem cells in skeletal muscle regeneration

Garza-Rodea, A.S. de la

Summary

Summary

Stem cell-based therapies represent promising approaches for the treatment of incurable diseases and tissue injury due to the capacity of these cells to self-renew and differentiate into specialized mature cells. Understanding the interaction of stem cells with the disease/injured environment and their contribution to the repair process by release of regeneration-promoting signals or by differentiation into the lost cell types is crucial for the advancement of their clinical application.

This thesis focuses on one stem cell population, the mesenchymal stem cells (MSCs) from human and in particular on their role and utility in skeletal muscle regeneration. For this purpose, several in vivo tissue damage models were employed. Most of the experiments were performed in immunocompromised mice (i.e. NOD/SCID) to avoid immunological rejection of the human cells.

MSC are attractive for cellular therapy of muscular dystrophies as they are easy to procure, can be greatly expanded ex vivo, and contribute to skeletal muscle repair in vivo. However, detailed information about contribution of bone marrow (BM)-derived human MSCs to skeletal muscle regeneration in vivo is very limited. In chapter 2, we investigated the participation of these cells in skeletal muscle regeneration following cardiotoxin-induced injury. The histological features of the damage induced by cardiotoxin injection in tibialis anterior muscle (TAM) is described. Then, the fate of locally injected *LacZ*-tagged human BM-MSCs was studied up to 4 months after damage. β-Galactosidase-positive (β-gal$^+$) human-mouse hybrid myofibers were counted in serial cross sections over the full length of the treated TAMs of groups of mice at monthly intervals. The number of hybrid myofibers increased from day 10 onwards, reaching 104±39.1 (5%) per TAM at 120 days post injection. Hybrid myofibers were further stained positive for human-muscle specific markers such as β-spectrin suggesting myogenic reprogramming of human MSC nuclei.

MSCs of mammals can be isolated from diverse tissues. Apart from the BM, adipose tissue (AT) and synovial membrane (SM) have been reported as useful sources of human MSCs. There are several studies describing their aptitude to contribute to skeletal muscle regeneration. However, the relative capacity of human MSCs of different origin to participate in skeletal muscle repair is difficult to determine as each cell source was investigated in isolation by different research groups. Additionally, for clinical applications of human MSCs, the acquisition, availability and amount of the tissue source are issues to be consider as limitation for cell therapy. We compared in a side-by-side

study in chapter 3, the myogenic properties of BM, SM and AT-MSCs derived from the same donors. In this way, inter individual and inter-laboratory variations were avoided and a fair comparison of the myoregenerative ability of human MSCs from different sources could be achieved both in vitro and in vivo. Our data show that human MSCs derived from the three tissues differ in phenotype, proliferation capacity and differentiation potential. The division rate of AT derived MSCs (AT-MSCs) was distinctly higher than that of SM- or BM-derived MSCs. Further, clear donor-specific differences in the long-term maintenance of MSC proliferation ability were observed. Although similar in their in vitro fusogenic capacity with murine myoblast, MSCs of the three sources contributed to a different degree to skeletal muscle regeneration in vivo. Transplanting *LacZ*-tagged human AT-, BM- or SM-MSCs into cardiotoxin-damaged TAMs of NOD/SCID mice revealed that at 30 days after treatment the frequency of hybrid myofibers was highest in the TAMs treated with AT-MSCs. Furthermore, human-specific β-spectrin-positive and dystrophin-positive hybrid myofibers containing human nuclei indicate myogenic programming of MSCs nuclei in regenerating murine skeletal muscle. In overall, this study provided evidence that AT-MSCs can be consider as the preferential source for clinical applications in myopathies.

Chapter 4 described the results of a study on the regeneration of the panniculus carnosus muscle (PCM) in mice following ischemia-reperfusion injury. For this purpose, pressure ulcers (PU) were induced in the dorsal skin of hairy, hairless, and diabetic mice by applying magnetic disks for different periods of time. No difference between hairy and hairless mice was observed in the healing rate of the PU. Surprisingly, healing was not delayed in diabetic mice. Therefore in an attempt to prolong tissue healing (chronic damage) PU were also induced in previously irradiated skin folds of mice. The healing process of all layers of the skin (i.e. epidermis, dermis and hypodermis -subcutis-) is described for each induction protocol. Pre-irradiation of the skin caused doubling of the time to complete closure of the PU and greatly delayed repair of dermis and PCM. The ischemia-reperfusion model was used further to study the contribution of human BM-MSCs to the regeneration of the PCM as well as to other resident cell types of the skin. In conclusion the intradermal transplantation of human BM-MSCs did not accelerate the healing and the grafted MSC only marginally participated in regeneration by differentiating into tissue specific cells, and these were short-lived. These results contrast with the therapeutic effects on MSC on surgical wounds and emphasize the unique properties of PU and its repair.

While standardizing conditions for PU induction and trying to set up models of chronic wound healing (e.g. diabetes, irradiation) we encountered large

variation in the effects of streptozotocin (STZ) resulting in unpredictable and low yields of diabetic mice. This agent is often used to induce diabetes in laboratory animals and are available in the literature several induction protocols. HPLC analysis has shown that the composition of the solution may change considerably during the first two hours after dissolution due to equilibration of the two anomers (α and β) of STZ. Because of the drug's alleged instability in solution, the typical recommendation is to administer STZ within ten minutes after dissolution. Chapter 5 reports a comparison of the induction of diabetes in NOD/SCID mice by injection of a single high dose of freshly made or anomer-equilibrated STZ solution. Both solutions induced long-term hyperglycemia, but blood glucose levels and mortality were higher and damage to pancreatic islands more pronounced in the mice receiving freshly prepared solution. The latter is due to the preponderance of the more toxic alpha anomer in the fresh solution. The anomer-equilibrated solution retains its biologic activity for at least forty days if stored at 4° C in the dark. All together these results suggest that the standard use of equilibrated STZ solutions has several practical advantages, will improves reproducibility and allows the results of different laboratories to be compared better.

Muscle regeneration and treatment of myodegeneration at present is mainly studied in animal muscles. The translational relevance of such models is unclear, especially regarding the curative properties of human stem and precursor cells. In those cases the issue of species specific interactions cannot be addressed. In vivo studies on human muscle are difficult due to practical and ethical issues and in vitro regeneration of muscle tissue has not been achieved so far. In chapter 6 are described preliminary findings of the use of subcutaneous implants of minced muscle tissue in immunodeficient mice which allow the comparison of syngeneic with xenogeneic combinations of therapeutic cells and damaged muscles. Myoregeneration of the implanted tissue in general was observed by immunohistology as early as day 7 that progresses with time, and proceed from the periphery of the implant inwards. The myoregeneration rate of human muscle tissue is slower than that of the murine muscle. We introduce cryopreservation of human muscle since there was irregular and insufficient supply of fresh tissue. However, this method shows to be toxic to the human satellite cells resulting in deficient myoregeneration. In contrast, regeneration of cryopreserved murine muscle implants was similar to that of the fresh muscle. *LacZ*-tagged human or murine MSC isolated both from BM that were mixed with the minced muscle prior to implantation were detected as single cells at the periphery of the implants at day 7. In both fresh and cryopreserved mouse muscle implants supplied with marked mouse MSC β-gal$^+$ hybrid myofibers were observed at 35 and 45 days after implantation.

Interestingly, similar hybrid myofibers appeared only when human MSC were added to cryopreserved mouse muscle, but not when mixed with fresh mouse muscle. This observation suggests that mouse muscle does not provide an optimal environment for human cell maintenance and fusion and that their contribution to regeneration may have been under evaluated in mouse models. This study required further development, and its usefulness will be significantly increased by devising an effective technique for the cryopreservation of satellite cells in human muscle. The morphological resemblance between the cryopreserved human muscle implants and the muscle tissue of DMD patients at a late stage of the disease makes further the frozen implants a candidate model for the study of muscle degeneration of the prospects of intervening with the progression of this process.

Repair of tissue damage that requires in situ differentiation of MSCs into specialized cell types or their fusion with resident cells has so far only been achieved with autologous/syngeneic MSCs or in immunocompromised recipients although these cells are held by many to be non-immunogenic. Similarly, successful use of MSCs as vehicles for the delivery of therapeutics depends on immunocompatible donor-recipient combinations. The involvement of surface MHC class I molecules in graft rejection and the effect of inhibiting MHC class I protein dependant recognition of the transplant on its immunogenicity have been well documented. In chapter 7, we described the downregulation of MHC class I protein expression on the surface of human BM-MSCs by retroviral vectors encoding a herpesviral immunoevasin (i.e. the US11). When transplanted into immunocompetent mice, persistence of the US11-expressing and HLA-ABC-negative human MSCs at levels resembling those found in immunodeficient NOD/SCID mice could be attained provided that recipients natural killer (NK) cells were depleted prior to cell transplantation. Those findings demonstrate the potential utility of herpesviral immunoevasins to prevent rejection of xenogeneic MSCs. The observation that downregulation of MHC class I surface expression renders human MSCs vulnerable to NK cell recognition and cytolysis implies that multiple viral immune evasion proteins are likely required to make human MSCs non-immunogenic and thereby universally transplantable.